

AMENDMENTS TO THE SPECIFICATION

Please delete the original Sequence Listing appearing on page 20-33 of the original specification.

Please amend the paragraph beginning on page 3, line 10 as follows:

The present inventors have searched enzymes which can attain the above purpose by estimating the three-dimensional structure of an alkaline cellulase having an amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Egl-237); in particular, the structure of its active domain, and by incorporating mutations through site-specific mutation. As a result, the present inventors have found that the optimum reaction pH in the CMC decomposition activity can be increased by deleting amino acid residues in a specific region which forms a portion of the loop structure and inserting a peptide into the position.

Please amend the paragraph beginning on page 3, line 21 as follows:

Accordingly, the present invention provides a mutated alkaline cellulase obtained by deleting, from a cellulase having an amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 or an amino acid sequence exhibiting at least 90% homology therewith, one or more amino acid residues chosen from the 343rd to 377th positions in ~~SEQ ID NO: 1~~ SEQ ID NO: 2 or from corresponding positions, and inserting a peptide having 2 to 15 amino acid residues into at least one of the deleted positions; as well as a gene encoding the mutated alkaline cellulase.

Please amend the paragraph beginning on page 4, line 8 as follows:

Figs. 1a to 1c show aligned amino acid sequences of cellulases having at least 90% homology with the amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Egl-

237). Egl-1139 appears as SEQ ID NO: 7, Egl-64 appears as SEQ ID NO: 8, and Egl-N131b appears as SEQ ID NO: 9.

Please amend the paragraph beginning on page 4, line 21 as follows:

The mutated alkaline cellulases according to the present invention are obtained by using, as a cellulase to be mutated (hereinafter may be referred to as "parent alkaline cellulase"), a cellulase having an amino acid sequence represented by in ~~SEQ ID NO: 1~~ SEQ ID NO: 2 or an amino acid sequence exhibiting at least 90% homology therewith, and by deleting one or more amino acid residues chosen from the 343rd to 377th positions in ~~SEQ ID NO: 1~~ SEQ ID NO: 2 or from corresponding positions and inserting a peptide having 2 to 15 amino acid residues into at least one of the deleted positions. The parent alkaline cellulases may be obtained either through spontaneous or artificial mutation of the cellulase having the amino acid sequence of ~~SEQ ID NO: 1~~ SEQ ID NO: 2.

Please amend the paragraph beginning on page 5, line 9 as follows:

The parent cellulase exhibiting 90% or more homology ~~With~~ with the amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 preferably exhibits 95% or more homology, more preferably 98% or more homology, with the amino acid sequence. The cellulase may be of wild-type or a mutant of a wild-type cellulase. The homology of an amino acid sequence can be calculated by means of a program such as maximum matching or search homology of GENETYX-WIN (Software Development Co.).

Please amend the paragraph beginning on page 5, line 17 as follows:

When the molecular structure of the cellulase exhibiting 90% or more homology with the amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 is estimated through a

homology modeling technique and by means of 3D-1D, XPLORE, and PROCHECK programs, the cellulase preferably has the following two characteristics; (i) the cellulase has an amino acid sequence exhibiting 70% or more homology, more preferably 80% or more homology, much more preferably 90% or more homology, still more preferably 95% or more homology, yet still more preferably 98% or more homology, with the region from the 42nd position (leucine) to the 404th position (valine) (i.e., the active domain region) (i.e., the active domain region) of ~~SEQ ID NO: 1~~ SEQ ID NO: 2; and (ii) the region from the 343rd position (asparagine) to the 377th position (leucine) of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 has a loop structure in the cellulase molecule. The homology of an amino acid sequence may be calculated in accordance with, for example, the Lipman-Pearson method (*Science*, 227, 1435, 1985).

Please amend the paragraph beginning on page 7, line 4 as follows:

Accordingly, the parent alkaline cellulase of the present invention is preferably, in addition to the alkaline cellulase having the amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2, an alkaline cellulase having (i) the above amino acid sequence features-i.e., having a high homology in the active domain region of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 and containing a particular region having a loop structure in the cellulase molecule as described above-and/or the above enzymatic characteristics (particularly preferably, having the amino acid sequence features and the enzymatic characteristics in combination), and (ii) an amino acid sequence exhibiting 90% or more homology (preferably 95% or more homology, much more preferably 98% or more homology) with that represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2.

Please amend the paragraph beginning on page 7, line 18 as follows:

Examples of the parent alkaline cellulase of the present invention include Egl-237 [derived from *Bacillus* sp. KSM-S237 (FERM BP-7875), which is "an alkaline cellulase having the amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2," Hakamada *et al.*, *Biosci. Biotechnol. Biochem.*, 64, 2281-2289, 2000]; alkaline cellulases derived from *Bacillus* sp. strain 1139 (Egl-1139; SEQ ID NO: 7) (Fukumori *et al.*, *J. Gen. Microbiol.*, 132, 2329-2335) (homology: 91.4%); alkaline cellulases derived from *Bacillus* sp. strain KSM-64 (Egl-64; SEQ ID NO: 8) (Sumitomo *et al.*, *Biosci. Biotechnol. Biochem.*, 56, 872-877, 1992) (homology: 91.9%); and cellulases derived from *Bacillus* sp. strain KSM-N131 (Egl-N131b; SEQ ID NO: 9) (Japanese Patent Application No. 2000-47237) (homology: 95.0%).

Please amend the paragraph beginning on page 8, line 4 as follows:

The mutated alkaline cellulase of the present invention is obtained by deleting, from the parent alkaline cellulase, one or more amino acid residues chosen from the 343rd to 377th positions in ~~SEQ ID NO: 1~~ SEQ ID NO: 2 or from corresponding positions and inserting a peptide having 2 to 15 amino acid residues into at least one of the deleted positions.

Please amend the paragraph beginning on page 8, line 10 as follows:

The amino acid residue(s) to be deleted may be any of 35 amino acid residues included in the 343rd to 377th positions of ~~SEQ ID NO: 1~~ SEQ ID NO: 2. The number of the amino acid residue(s) to be deleted may be any of 1 to 35. The amino acid residues to be deleted are continuous or non-continuous. The amino acid residue(s) to be deleted is(are) preferably included in the 350th to 377th positions, more preferably in the 355th to 365th positions, much more preferably in the 357th to 362nd positions, of ~~SEQ ID NO: 1~~ SEQ ID NO: 2.

Please amend the paragraph beginning on page 8, line 27 as follows:

Three-dimensional structural analysis through homology modeling (Ozawa *et al.*, *Protein Eng.*, 14, 501-504, 2001) suggests that the amino acid region at the 343rd to 377th positions of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 is located relatively distant from the active center of Egl-237 and therefore has a high degree of freedom, and is suggested to be a region that forms a portion of the loop structure that is intimately involved in maintaining the cellulase structure.

Please amend the paragraph beginning on page 9, line 8 as follows:

The "amino acid residue corresponding to the 343rd to 377th positions of ~~SEQ ID NO: 1~~ SEQ ID NO: 2" can be identified by comparing amino acid sequences by means of a known algorithm such as Lipman-Pearson's method, and aligning the amino acid residues contained in the amino acid sequences of the alkaline cellulases such that the homology of each amino acid sequence with respect to that of ~~SEQ ID NO: 1~~ SEQ ID NO: 2 is maximized. By aligning the amino acid sequence of the cellulase in such a manner, the position of the homologous amino acid residue in the amino acid sequence of each cellulase can be determined, irrespective of insertion or deletion in the amino acid sequence (Fig. 1). The homologous position is presumed to exist at the same three-dimensional position and to bring about similar effects with regard to a specific function of the target cellulase.

Please amend the paragraph beginning on page 9, line 23 as follows:

Table 1 shows the positions of Egl-1139 (SEQ ID NO: 7), Egl-64 (SEQ ID NO: 8), and Egl-N131b (SEQ ID NO: 9) corresponding to the 357th to 362nd positions of alkaline cellulase having an amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2 (Egl-237).

Please amend the paragraph beginning on page 11, line 15 as follows:

Specifically, a parent alkaline cellulase is cultured, and the resultant culture broth is centrifuged, to thereby isolate cells. Through use of the alkaline cellulase gene collected from the cells, a chromosomal DNA containing the alkali cellulase gene is prepared [through, for example, a method of Marmar (*J. Mol. Biol.*, 3, 208-212, 1961) or a method of Saito and Miura (*Biochim. Biophys. Acta*, 72, 619-629, 1963)]. The chromosomal DNA may be subjected to cloning through shotgun cloning or PCR, to thereby prepare a gene (~~SEQ ID NO: 2~~ SEQ ID NO: 1) encoding the parent alkaline cellulase (e.g., alkaline cellulase having an amino acid sequence represented by ~~SEQ ID NO: 1~~ SEQ ID NO: 2). To the thus-obtained gene, a mutation is introduced, and the resultant mutated gene is incorporated to a plasmid. Appropriate host cells are transformed through use of the plasmid and then cultured, and the mutated alkaline cellulase of the present invention may be collected from the culture.

Please delete the original Abstract (page 36) and insert therefor the attached substitute Abstract as new page 36.

Page 36 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.